CHIRAL ANALYSIS OF AMINO ACIDS USING COMPOSITE BIENZYME BIOSENSORS

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Amino acids are essential compounds for life, and are ubiquitously found in nature, their analysis being interesting in many fields. For example, the chiral evaluation of amino acids in food samples such as wines, beers and vinegars (1) is important in order to determine the quality and the origin of the product. Moreover, the chiral amino acids profile is a useful tool for the monitoring of many fermentation processes, and it is also an estimation of the presence of bacterial activity (2). Their analysis is also employed in biogeochemistry, and the degree of racemization of the L- and D-isomers can be used to estimate the age of the sample (3). There are also some clinical applications that include monitoring of some metabolic disorders caused by several diseases in which some fails in the metabolism of amino acids are associated.

The chiral analysis of amino acids can be carried out using a chiral thiol compound to form diastereomeric derivatives, which can be separated by chromatography on a stationary phase. It is also possible to use chiral stationary phases or chiral mobile phases (4). Another approach relies on the enzymatic reactions of the L- and D-amino acid oxidases, respectively; several biosensor designs in which these enzymes were immobilised on different electrode configuration have been reported (5,6).

In this communication, the construction of composite graphite-Teflon electrodes, in which the enzymes D- or L-amino acid oxidases (AAOD) and peroxidase (HRP) are coimmobilised, together with the mediator ferrocene, as well as their performance for the analysis of D- and L-amino acids is described. These composite electrodes are constructed by simple physical inclusion of the enzymes into the bulk of a graphite-Teflon pellet with no covalent attachments, which makes the electrode fabrication procedure easier, faster and cheaper, and avoids possible losses in sensitivity due to the covalent linkages. Furthermore, this type of composite electrodes gives rise to a three-dimensional rigid biocomponents reservoir whose surface can be easily regenerated by polishing.

The composite L- and D-bienzyme electrodes showed a well-defined amperometric response for successive additions of the L-and D-amino acids tested (L-tryptophan, L-arginine, L-phenylalanine, L-methionine, L-leucine, D-methionine, D-leucine, D-serine and D-valine). Coimmobilization of L- or D- AAOD with HRP and ferrocene allowed the use of 0.0 V as the detection potential, thus minimising interferences from other electroactive compounds present in the samples. The selected working medium was 0.05 mol L^{-1} phosphate 9.0.

Figure 1 shows the control chart constructed for an L-AAOD-HRP-ferrocene-graphite-Teflon electrode using L-tryptophan as the substrate. When a mean value of three measurements performed everyday was out of the lower limit of control, the electrode surface was polished for some seconds and the initial signal could be restored. After approximately 30 days, the initial amperometric response could not be recovered by polishing. The reproducibility of the analytical signal obtained with different electrodes (n=3) was good with RSD values lower than 7%.

The good stability and fast response exhibited by the amino acid bioelectrodes allowed their use in flow-injection systems, in connection with amperometric detection. Under these conditions, linear ranges between approximately $5.0x10^{-6}$ and $1.0x10^{-3}$ mol L^{-1}, were obtained. The limits of detection for the amino acids tested are around 10^{-6} mol L^{-1} which shows an improved sensitivity of the biosensor design when compared to literature data.

Moreover, these electrodes can be used as suitable detectors in a series configuration under HPLC conditions, which would make possible the separation and detection of L- and D-amino acids with no need of using derivatization procedures or chiral phases.

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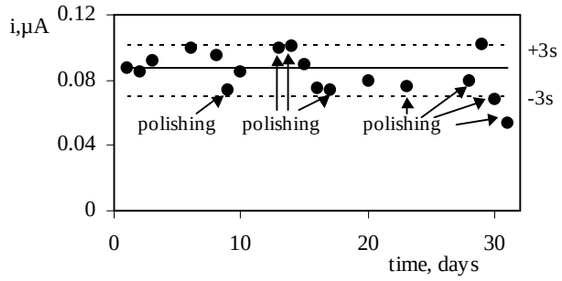

Figure 1.- Control chart for the steady-state current dependence on time for $4.0x10^{-4}$ mol L^{-1} of L-tryptophan measured at a graphite-Teflon-L-AAOD-HRP-ferrocene electrode. Phosphate buffer (pH 9.0). E_{app}=0.0 V.